

# Synovial C-Shaped Tibial Footprint of the Anterior Cruciate Ligament



César Janovsky,<sup>\*†</sup> MD, Camila Cohen Kaleka,<sup>‡</sup> MD, Maria Teresa Seixas Alves,<sup>§</sup> MD, PhD, Mario Ferretti,<sup>†‡</sup> MD, PhD, and Moises Cohen,<sup>†‡</sup> MD, PhD

*Investigation performed at Sports Orthopedic Trauma Center, Orthopedic Department, Federal University of São Paulo, UNIFESP, São Paulo, Brazil*

**Background:** Although numerous anatomic studies about the anterior cruciate ligament (ACL) structure and attachments have been performed, these studies have not reached consensus on the ACL footprint.

**Purpose:** To investigate the existing controversy regarding the morphology of the tibial ACL insertion (footprint) and confirm histologically that the tibial ACL footprint is not completely filled with ligament tissue.

**Study Design:** Descriptive laboratory study.

**Methods:** The tibial ACL footprint was dissected from 20 different fresh-frozen cadaveric knees (all males; mean age, 68.8 ± 5.4 years [range, 55-80 years]; mean weight, 78 ± 6.6 kg [range, 45-93 kg]). Two knees, 1 with severe osteoarthritis and 1 with previous knee surgery, were excluded. The tibial ACL insertion was observed, and this area was longitudinally divided into 4 parallel slices (0%-25%, 25-50%, 50%-75%, and 75%-100%), embedded in paraffin wax, and stained with hematoxylin-eosin, alcian blue, and picrosirius-polarization. The specimens were measured using a microscope to determine the distances from the anterior to the posterior border of the ACL ligament tibial insertion and the distance from the posterior border to the end of the ligament fibers of the ACL ligament tibial insertions.

**Results:** The 18 evaluated knee specimens confirmed the finding of a C-shaped tibial insertion of the ACL. The measurements showed that the ligament (vertical parallel collagen fibers) occupied only 30.8% of the complete insertion. The remaining area was filled with synovial tissue, demonstrating histologically the “C” shape.

**Conclusion:** This study confirms macroscopically the C-shaped tibial insertion of the ACL and shows histologically that synovial tissue is an indirect insertion filling the major part of the footprint.

**Clinical Relevance:** This anatomic study suggests a different shape of the ACL tibial footprint, which may be useful for new perspectives regarding ACL reconstruction surgery research.

**Keywords:** knee; ACL; ligament; anatomy

The anterior cruciate ligament (ACL) is currently being intensively studied, and its surgical reconstruction after lesion is one of the most common procedures performed by orthopaedic surgeons.<sup>5,11</sup> Although numerous anatomic studies about the ACL structure and attachments have

been performed, these studies have not reached consensus regarding the ACL footprint.<sup>7,23,25,29</sup>

Many anatomic and biomechanical ACL studies have confirmed the existence of 2 bundles and an oval-shaped footprint. Other anatomic dissections suggest that there are more bundles that are less important than the 2 major functional ones: the anteromedial (AM) and posterolateral (PL).<sup>1-3,12,18,22,24</sup> However, histological studies remain controversial: Some find no evidence of different anatomic subdivisions of the ACL, whereas others show a well-defined septum dividing the AM and PL bundles.<sup>4,9,21,25,28</sup>

Recently, these bundles have been described as flat instead of cylindrical.<sup>14,15,17</sup> Additionally, anatomic dissections suggest that the ACL footprint is C-shaped and not completely filled with ligament tissue, as previously thought.<sup>14,24</sup> This finding is very important for knee surgery aiming at a more anatomic and functional ACL reconstruction. However, it is not clear which structures fill or

\*Address correspondence to César Janovsky, MD, Sports Orthopedic Trauma Center, Orthopedic Department, Federal University of São Paulo, UNIFESP, São Paulo, Brazil (email: cesar.janovsky@gmail.com).

†Sports Orthopedic Trauma Center, Orthopedic Department, Federal University of São Paulo, UNIFESP, São Paulo, Brazil.

‡Hospital Israelita Albert Einstein, São Paulo, Brazil.

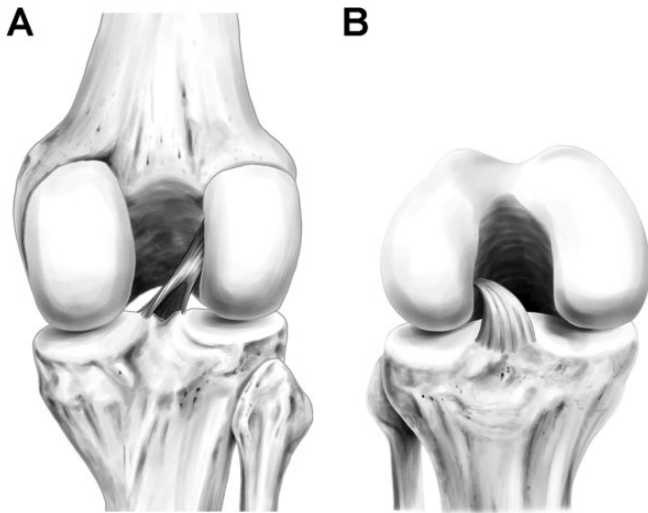
§Department of Pathology, Federal University of São Paulo, UNIFESP, São Paulo, Brazil.

The authors declared that they have no conflicts of interest in the authorship and publication of this contribution.

The Orthopaedic Journal of Sports Medicine, 4(11), 2325967116671300

DOI: 10.1177/2325967116671300

© The Author(s) 2016



**Figure 1.** (A) Posterior view of the anterior cruciate ligament (ACL). The ACL tibial footprint has a “C” shape. (B) Anterior view of the ACL.

are present on the footprint area besides the “C-shaped” ligament tissue.

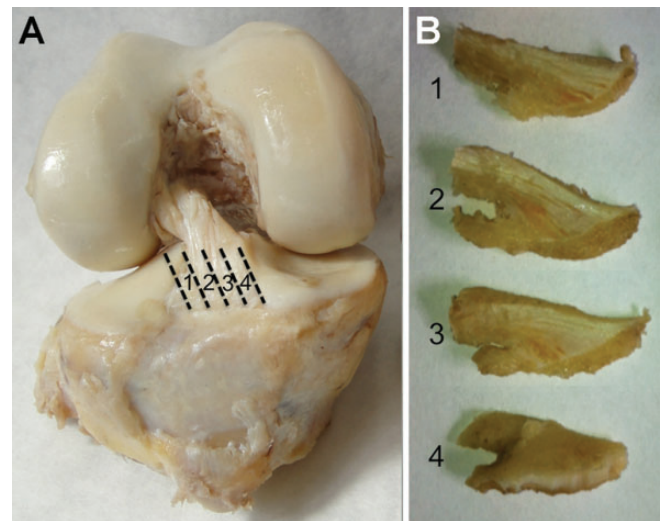
Therefore, the purpose of this anatomic study was to investigate the existing controversy regarding the morphology of the tibial ACL insertion (footprint) and confirm histologically the C-shape of the ACL footprint (Figure 1).<sup>24</sup>

## METHODS

This study was approved by an ethics committee and was conducted according to the ethical standards of the Declaration of the World Medical Association Helsinki.

We evaluated 20 different fresh-frozen cadaveric knees for this study (all males, separate donors; mean age,  $68.8 \pm 5.4$  years [range, 55-80 years]; mean weight,  $78 \pm 6.6$  kg [range, 45-93 kg]). The knees were removed from cadavers after autopsy and family authorization, within 7 to 48 hours from death. Two knees, 1 with severe osteoarthritis and 1 with previous knee surgery, were excluded. The soft tissue structures around the knees were removed to expose the joint. An oscillating saw was used to remove the ACL ligament with the bone footprints (femur and tibia) to prevent any damage to the insertion or superficial fibrous membrane covering the middle substance of the ACL and ACL insertions (femur and tibia). The entire tibial ACL footprint was dissected carefully by the same orthopaedic surgeon.

The cadaveric specimens were fixed in formalin solution (10% neutral-buffered) for 2 days, followed by decalcification with  $\text{HNO}_3$  (1/1 vol/vol) for 72 hours regardless of the bone quality. The histological evaluation began with surgical ink–signaling of the medial and lateral limits of the ACL footprint, which were observed macroscopically, and these areas were longitudinally divided (oblique divisions of anatomic view and parallel to the footprint axis) into 4



**Figure 2.** (A) Anterior view of the anterior cruciate ligament (ACL). (B) The footprint area was divided into 4 parallel slices for histologic analysis: 1 (0%-25%), 2 (25%-50%), 3 (50%-75%), and 4 (75%-100%).

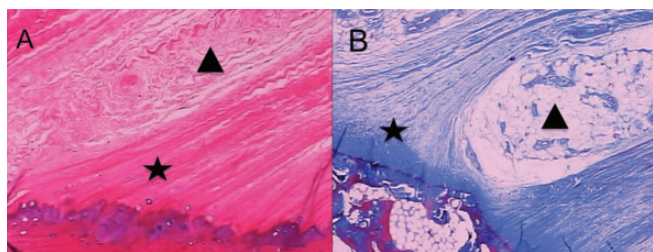
parallel slices from lateral to medial (0%-25%, 25%-50%, 50%-75%, and 75%-100%) of the full macroscopic insertion (Figure 2). Afterward, the blocks were embedded in paraffin wax and sliced into four 5- $\mu\text{m}$ -thick sections. The sections that were closer to the insertion site were then stained with hematoxylin and eosin (H&E) (Figure 3A) and alcian blue at pH 2.5 (Figure 3B). H&E was used to observe the morphology of the ACL insertion and alcian blue to enhance collagen-containing fibers (nonspecific type of collagen).<sup>4</sup>

The ACL insertions were carefully observed with a microscope (Olympus) at  $40\times$ ,  $100\times$ , and  $400\times$  magnifications. All blocks were also analyzed by comparing the ultrastructural picture with the results obtained using the specific method for collagen-containing fibers (picrosirius-polarization) by light microscopy. Quantification of collagen-type concentration was made using the  $400\times$  scale. Collagen type 1 appears in red, and type 3 is green.

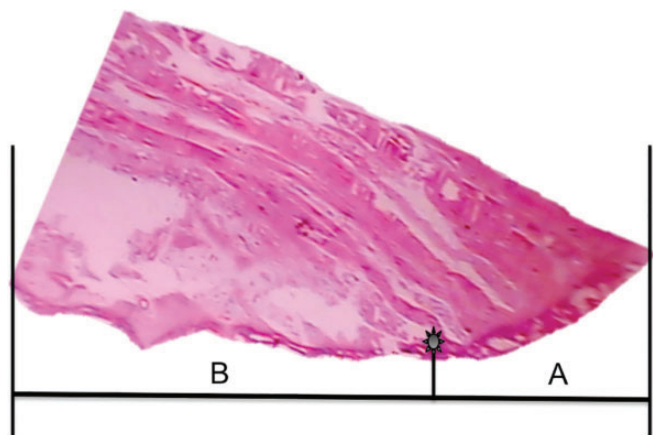
Next, the specimens were measured using the microscope (Olympus) at  $40\times$  to determine the length of macroscopic tibial footprint from the anterior to posterior border of the ACL ligament tibial insertion (length “A” in Figure 4) and the distance from the posterior border to the end of the ligament fibers (length “B” in Figure 4).

## Statistical Analysis

There were no comparison groups. Median and range were calculated for all specimens. Standard deviation and deviation from the normal distribution were calculated for all variables using the Kolmogorov-Smirnov test. Morphometric measurements were described by descriptive analysis.



**Figure 3.** Histological aspect of the laminae (parallel slices): (A) hematoxylin-eosin and (B) alcian blue staining. Note that the ligament fibers (star) are present with a synovial tissue (triangle) in the entire aspect of the ligament fibers.



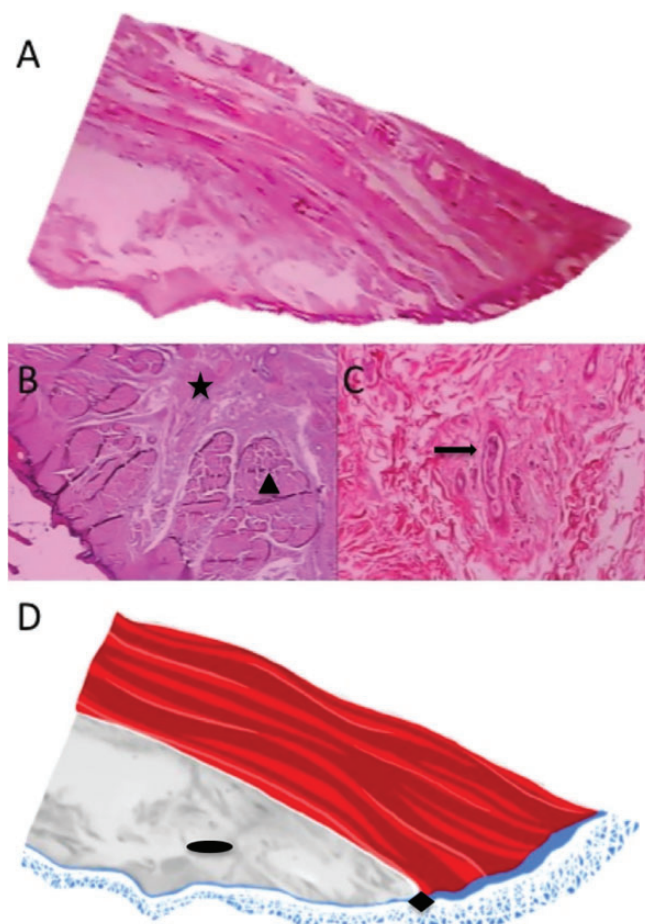
**Figure 4.** Microscopic measurements: (A and B) distance of the entire macroscopic footprint, (A) distance of the ligament insertion, and (B) distance of the synovial tissue insertion.

## RESULTS

The 18 evaluated knee specimens confirmed the finding of a C-shaped tibial insertion of the ACL. In addition to the “C” shape, there was a rotation of insertion area between the medial and lateral intercondylar tubercles, with the ACL pointing toward the femoral insertion region. The microscopic evaluation showed a clear division between the ACL (vertical parallel collagen fibers) and the synovial tissue (Figure 5, A and B). Another important finding was the presence of histological bundles divided by the same synovial tissue found inside the ACL insertion area, which comprises neurovascular structures as well as arterial and venous bundles (Figure 5, B and C). We found that the insertion area ligament-bone was composed of ligament tissue, noncalcified fibrocartilage, and calcified fibrocartilage (Figure 6).

The measurements showed that the ligament (vertical parallel collagen fibers) occupied only 30.8% of the complete insertion (Table 1). The remaining area was filled with synovial tissue (see Figure 4).

The polarized light microscopic analysis with picrosirius showed a higher concentration of type 1 collagen in



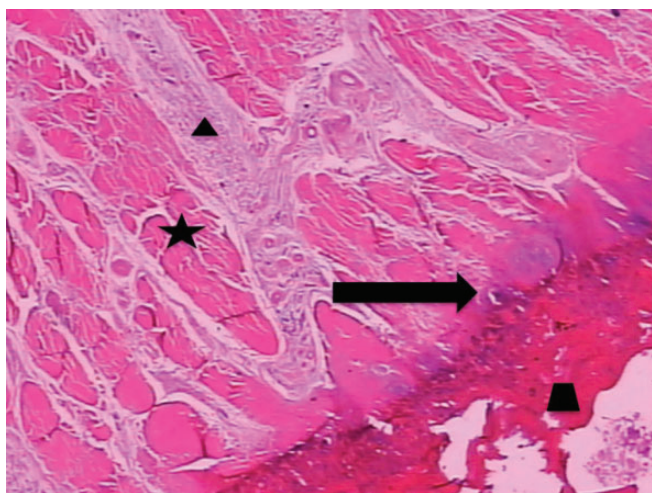
**Figure 5.** (A) Anterior cruciate ligament (ACL) insertion in a hematoxylin-eosin-stained histological sample. Many bundles of ACL ligament and the posterior aspect of the synovial tissue are shown. (B) Axial histological view of the synovial tissue (star) and ligament bundles (triangle) (100× original magnification). (C) Neurovascular structures (arrow) (400× original magnification). (D) Limit of the ligament/synovial tissue (diamond) and synovial tissue (oval). The red represents the ligament tissue, gray represents the synovial tissue, and the wide blue line represents bone tissue.

the ligament fibers, which was in contrast to the higher type 3 collagen concentration observed in the posterior area of the ligament fibers.<sup>4</sup> The synovial tissue between the ACL bundles was similar to the posterior area tissue rather than the previously expected similarity to the ligament (Figure 7).

## DISCUSSION

ACL reconstruction is undoubtedly one of the most common procedures in orthopaedic practice. Thus, studies regarding the function and morphology of the ACL are continuously being sought. Based on routine arthroscopic surgery, anterior to posterior observations of the ACL, and innumerable past publications, we have believed that





**Figure 6.** Insertion area ligament-bone (100 $\times$ ) showing non-calcified fibrocartilage (arrow), ligament fibers (star), synovial tissue (triangle), and calcified fibrocartilage (trapezoid).

the ACL presents as a solid and cylindrical structure.<sup>17</sup> However, after recent studies,<sup>5,7,11,23,29</sup> the idea of a functionally cylindrical ligament with a flat morphology has gained attention.

The introduction of ACL reconstruction with a double bundle has promoted the study of tibial and femoral insertions, mainly by examining the anatomic region more closely.<sup>11</sup> Anatomic studies using cadaver dissection are unparalleled sources of knowledge.<sup>4</sup> The empty space in the tibial insertion of the ACL region was present in all dissections. The ligament footprint within this space was found to be C-shaped, oriented like a “C” written along the anterior-posterior tibial axis in an anterior to posterior direction (see Figure 1).<sup>6</sup> Starting from the tibia within a large and hollow shape, the ligament heads to the femur, modifying its shape and transforming itself into a thin cylindrical structure before inserting into the lateral intercondylar ridge (“resident’s ridge”).<sup>10,14,15,17,24</sup>

Histological slices, which were transverse to the ligament, showed an expressive difference between the ligament and the synovial tissue, as seen in Figures 2, 3, and 5. Macroscopically, the difference between the ligament region and the synovial tissue were easily detected. The search for ACL reconstruction methods that best preserve the actual anatomic position justifies and highlights our findings. With those findings, it may be possible to propose methods and techniques to reproduce the C-shaped tibial insertion.

Picrosirius polarization analysis is widely used in histochemical studies that assess collagen and was chosen because it is easy to manage, reproducible, and cost effective.<sup>20,24</sup> Immunohistochemistry femoral footprint research has shown a collagen distribution pattern

**TABLE 1**  
Microscopic Observation of the Anterior Cruciate Ligament Tibial Footprint

|               | Ligament Insertion, mm | Synovial Tissue Insertion Fibers on Tibial Footprint, mm |
|---------------|------------------------|--|
| Knee specimen |                        |  |
| 1             | 5.15                   | 12.08  |
| 2             | 5.02                   | 11.43  |
| 3             | 4.73                   | 12.83  |
| 4             | 5.12                   | 12.7   |
| 5             | 4.74                   | 11.71  |
| 6             | 5.87                   | 12.17  |
| 7             | 4.56                   | 10.99  |
| 8             | 5.75                   | 10.03  |
| 9             | 4.81                   | 13.08  |
| 10            | 5.37                   | 11.41  |
| 11            | 4.98                   | 9.75   |
| 12            | 5.21                   | 11.37  |
| 13            | 4.04                   | 13.48  |
| 14            | 5.29                   | 11.05  |
| 15            | 5.11                   | 10.38  |
| 16            | 4.98                   | 11.5   |
| 17            | 5.49                   | 10.41  |
| 18            | 6.23                   | 11.26  |
| Mean $\pm$ SD | 5.1361 $\pm$ 0.5056    | 11.535 $\pm$ 1.0178                                      |

similar to our study.<sup>23</sup> The type 1/type 3 collagen ratio found in the ligament at the area between the bundles and the posterior region of the ligament gradually decreased, with a greater concentration of type 3 collagen at the posterior region.

Some ACL studies use the *direct and indirect fibers* concept terminology.<sup>23,24</sup> These different fibers are presented as separate parts of the ligament. In our study, the ligament insertion can be addressed as direct fibers and the synovial tissue insertion as indirect fibers. Different from the previous study on the ACL tibial footprint “duck foot,” where the indirect fibers were described as being located anterior to the direct fibers, our findings showed ligament direct fiber insertion to be anterior to synovial tissue indirect fiber insertion.<sup>24</sup>

Although not part of the ligament, the tissue found posterior to the ACL ligament, in the central area of the “C,” has a primordial function as the origin of the connective tissue between the bundles of the ligaments that support the ACL vascular-nervous structures. These findings can be applied to recently described remnant preservation ACL reconstruction techniques.<sup>8,16,19</sup>

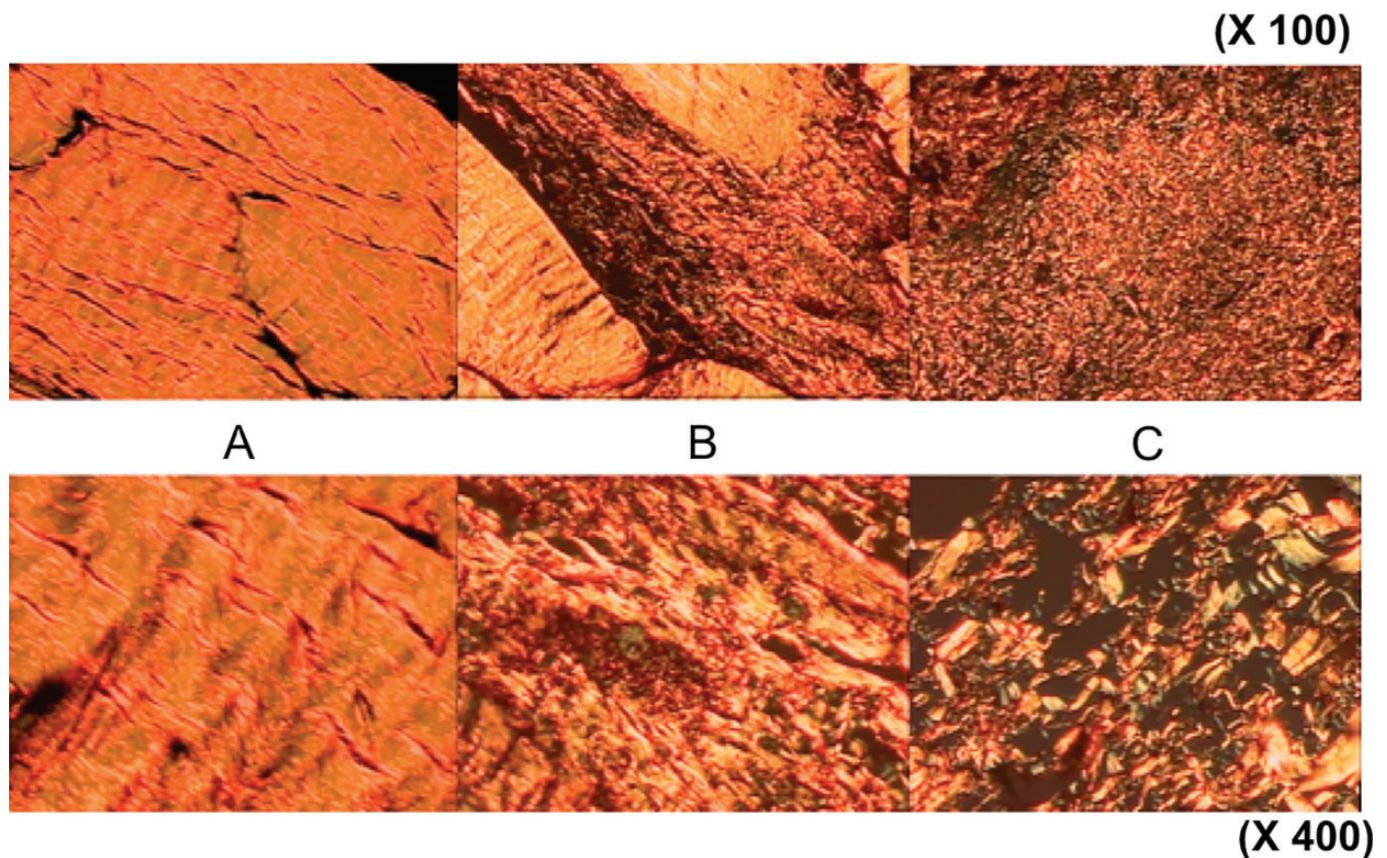
Once the C-shaped format is widely accepted, future studies might suggest reconstructions where the perforation is made only on the region without ligament tissue (the internal part of the “C”).

Double-bundle reconstruction has been used worldwide as a more functional reconstruction of the ACL.<sup>5,7,13,27,29</sup> After these findings, this type of reconstruction earns even more credibility, since both the AM and PL bundles are represented in the C-shaped concept (Figure 8).

<sup>11</sup>References 1-3, 5, 7, 12, 18, 22, 23, 25, 26, 29.

<sup>4</sup>References 1-3, 6, 9, 12, 18, 21, 22, 24, 25, 28.

<sup>6</sup>References 4, 9, 14, 15, 17, 21, 24, 25, 28.



**Figure 7.** Picrosirius polarization analysis by light microscopy (100 $\times$  and 400 $\times$  magnification). Collagen type 1 appears in red and type 3 is green at the ligament area. The ligament region shows a great concentration of collagen type 1. (A) Image of the ligament region showing a great concentration of collagen type 1. (B) Overview of the area between the bands showing a greater amount of collagen type 3 than observed in the ligament region. (C) Image of the posterior ligament showing a large amount of collagen type 3.



**Figure 8.** The posterior aspect of the C-shaped anterior cruciate ligament insertion showing the anteromedial (AM) and posterolateral (PL) bundles with the knee extended.

The major limitation of this study includes the age of the specimens in that advanced age could provide tissue degeneration and contribute to the C-shaped formation. Future studies in other age groups are needed to clarify this issue. Additionally, immunohistochemical analysis could have been used to assess the collagen; however, we obtained a thorough analysis and expected results with picrosirius polarization analysis. New perspectives about ACL

reconstruction may be available in future studies, including new methods on tibial drilling (small drills, curve osteotomies, or new guides systems) and graft shapes to reproduce the ACL “C-shaped” insertion as accurately as possible.

## CONCLUSION

Our study confirmed macroscopically the ACL C-shaped tibial insertion and showed histologically that synovial tissue is an indirect insertion filling the major part of the footprint (see the Video Supplement).

A Video Supplement for this article is available at <http://ojsm.sagepub.com/supplemental>.

## REFERENCES

1. Amis AA. The functions of the fibre bundles of the anterior cruciate ligament in anterior drawer, rotational laxity and the pivot shift. *Knee Surg Sports Traumatol Arthrosc.* 2012;20:613-620.

2. Amis AA, Dawkins GP. Functional anatomy of the anterior cruciate ligament. Fibre bundle actions related to ligament replacements and injuries. *J Bone Joint Surg Br.* 1991;73:260-267.
3. Arnoczky SP. Anatomy of the anterior cruciate ligament. *Clin Orthop Relat Res.* 1983;172:19-25.
4. Battlehner CN, Carneiro Filho M, Ferreira Júnior JM, Saldiva PH, Montes GS. Histochemical and ultrastructural study of the extracellular matrix fibers in patellar tendon donor site scars and normal controls. *J Submicrosc Cytol Pathol.* 1996;28:175-186.
5. Buoncristiani AM, Tjoumakaris FP, Starman JS, Ferretti M, Fu FH. Anatomic double-bundle anterior cruciate ligament reconstruction. *Arthroscopy.* 2006;22:1000-1006.
6. Cohen M, da Costa Astur D, Kaleka CC, et al. Introducing 3-dimensional stereoscopic imaging to the study of musculoskeletal anatomy. *Arthroscopy.* 2011;27:593-596.
7. Crawford C, Nyland J, Landes S, et al. Anatomic double bundle ACL reconstruction: a literature review. *Knee Surg Sports Traumatol Arthrosc.* 2007;15:946-964.
8. da Silveira Franciozi CE, Ingham SJ, Gracitelli GC, Luzo MV, Fu FH, Abdalla RJ. Updates in biological therapies for knee injuries: anterior cruciate ligament. *Curr Rev Musculoskelet Med.* 2014;7:228-238.
9. Dienst M, Burks RT, Greis PE. Anatomy and biomechanics of the anterior cruciate ligament. *Orthop Clin North Am.* 2002;33:605-620.
10. Ferretti M, Ekdahl M, Shen W, Fu FH. Osseous landmarks of the femoral attachment of the anterior cruciate ligament: an anatomic study. *Arthroscopy.* 2007;23:1218-1225.
11. Ferretti M, Levicoff EA, Macpherson TA, Moreland MS, Cohen M, Fu FH. The fetal anterior cruciate ligament: an anatomic and histologic study. *Arthroscopy.* 2007;23:278-283.
12. Girgis FG, Marshall JL, Monajem A. The cruciate ligaments of the knee joint. Anatomical, functional and experimental analysis. *Clin Orthop Relat Res.* 1975;106:216-231.
13. Giron F, Cuomo P, Edwards A, Bull AM, Amis AA, Aglietti P. Double-bundle "anatomic" anterior cruciate ligament reconstruction: a cadaveric study of tunnel positioning with a transtibial technique. *Arthroscopy.* 2007;23:7-13.
14. Hara K, Mochizuki T, Sekiya I, Yamaguchi K, Akita K, Muneta T. Anatomy of normal human anterior cruciate ligament attachments evaluated by divided small bundles. *Am J Sports Med.* 2009;37:2386-2391.
15. Iwahashi T, Shino K, Nakata K, et al. Direct anterior cruciate ligament insertion to the femur assessed by histology and 3-dimensional volume-rendered computed tomography. *Arthroscopy.* 2010;26(9 suppl):S13-S20.
16. Kondo E, Yasuda K, Onodera J, Kawaguchi Y, Kitamura N. Effects of remnant tissue preservation on clinical and arthroscopic results after anatomic double-bundle anterior cruciate ligament reconstruction. *Am J Sports Med.* 2015;43:1882-1892.
17. Mochizuki T, Muneta T, Nagase T, Shirasawa S-I, Akita K-I, Sekiya I. Cadaveric knee observation study for describing anatomic femoral tunnel placement for two-bundle anterior cruciate ligament reconstruction. *Arthroscopy.* 2006;22:356-361.
18. Morgan CD, Kalman VR, Grawl DM. Definitive landmarks for reproducible tibial tunnel placement in anterior cruciate ligament reconstruction. *Arthroscopy.* 1995;11:275-288.
19. Muneta T, Koga H, Nakamura T, Horie M, Watanabe T, Sekiya I. Behind-remnant arthroscopic observation and scoring of femoral attachment of injured anterior cruciate ligament. *Knee Surg Sports Traumatol Arthrosc.* 2016;24:2906-2914.
20. Nagai M, Aoyama T, Ito A, et al. Alteration of cartilage surface collagen fibers differs locally after immobilization of knee joints in rats. *J Anat.* 2015;226:447-457.
21. Odensten M, Gillquist J. Functional anatomy of the anterior cruciate ligament and a rationale for reconstruction. *J Bone Joint Surg Am.* 1985;67:257-262.
22. Petersen W, Tillmann B. Anatomy and function of the anterior cruciate ligament [in French]. *Orthopade.* 2002;31:710-718.
23. Sasaki N, Ishibashi Y, Tsuda E, et al. The femoral insertion of the anterior cruciate ligament: discrepancy between macroscopic and histological observations. *Arthroscopy.* 2012;28:1135-1146.
24. Siebold R, Schuhmacher P, Fernandez F, et al. Flat midsubstance of the anterior cruciate ligament with tibial "C-"shaped insertion site. *Knee Surg Sports Traumatol Arthrosc.* 2015;23:3136-3142.
25. Tállay A, Lim M-H, Bartlett J. Anatomical study of the human anterior cruciate ligament stump's tibial insertion footprint. *Knee Surg Sports Traumatol Arthrosc.* 2008;16:741-746.
26. Takeda Y, Iwame T, Takasago T, et al. Comparison of tunnel orientation between transtibial and anteromedial portal techniques for anatomic double-bundle anterior cruciate ligament reconstruction using 3-dimensional computed tomography. *Arthroscopy.* 2013;29:195-204.
27. van Eck CF, Lesniak BP, Schreiber VM, Fu FH. Anatomic single- and double-bundle anterior cruciate ligament reconstruction flowchart. *Arthroscopy.* 2010;26:258-268.
28. Zantop T, Petersen W, Sekiya JK, Musahl V, Fu FH. Anterior cruciate ligament anatomy and function relating to anatomical reconstruction. *Knee Surg Sports Traumatol Arthrosc.* 2006;14:982-992.
29. Zhao J, He Y, Wang J. Double-bundle anterior cruciate ligament reconstruction: four versus eight strands of hamstring tendon graft. *Arthroscopy.* 2007;23:766-770.